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Research note

First record of *Batrachochytrium dendrobatidis* infecting threatened populations of Tandilean Red-belly toad (*Melanophryniscus* aff. *montevidensis*) in Argentina

Primer registro de Batrachochytrium dendrobatidis *infectando poblaciones amenazadas del sapito de panza roja de Tandil (*Melanophryniscus *aff.* montevidensis*) en Argentina*

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Abstract

We present the first record of *Batrachochytrium dendrobatidis* (*Bd*) infecting endangered populations of the Tandilean Red-belly toad (*Melanophryniscus* aff. *montevidensis*). We obtained skin swab samples of 32 individuals. The prevalence was 35.5% and the infection levels varied between 0.34 and 915 *Bd*-genomic equivalents. This finding represents a new threat that could be affecting, in conjunction with the high habitat fragmentation, the viability of the studied populations.

Keywords: Chytridiomycosis; Amphibians; Population decline; Highland grassland

Resumen

Presentamos el primer registro de *Batrachochytrium dendrobatidis* infectando poblaciones amenazadas del sapito de panza roja de Tandil (*Melanophryniscus* aff. *montevidensis*). Obtuvimos muestras de hisopados de piel de 32 individuos cuyos resultados indicaron una prevalencia del 35.5% y niveles de infección que variaron entre 0.34-915 equivalentes genómicos de *Batrachochytrium dendrobatidis*. Este hallazgo representa una nueva amenaza que, conjuntamente con la alta fragmentación del hábitat, podría estar afectando la viabilidad de las poblaciones estudiadas.

Palabras clave: Quitridiomicosis; Anfibios; Declinación poblacional; Pastizales de altura

Chytridiomycosis is an emerging infectious disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which has contributed to amphibian population declines and extinctions worldwide (Blaustein et al., 2011). In Argentina, up to 16 species from different biogeographic regions (e.g., Paranaense, Patagonica, Pampeana, del Monte, Chaqueña,

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Espinal provinces) have been positive for *Bd* (Ghirardi, Perotti, Steciow, Arellano, & Natale, 2011; Lescano, Longo, & Robledo, 2013). Few studies have been conducted on threatened species; *Telmatobius pisanoi*, *T. atacamensis* (Barrionuevo & Mangione, 2006), and *Atelognathus patagonicus* (Fox, Greer, Torres-Cervantes, & Collins, 2006) were positive for *Bd*, but none of these studies conclude that chytridiomycosis is responsible for the population declines.

The Red-belly toad belongs to the *Melanophryniscus stelzneri* group. The taxonomy of the species in this group has not yet been adequately resolved (Kwet, Maneyro, Zillikens, & Mebs, 2005). Consequently, we refer to the population that inhabits Tandilean and Ventania mountains ridges as *Melanophryniscus* aff. *montevidensis* (Vaira et al., 2012). However, it is important to note that Ventania and Tandilia populations are isolated and they are probably different lineages.

The Tandilean Red-belly toad is an endemic species restricted to the remnants of highland grasslands in the Tandilean Mountains (Soler, Cortelezzi, Berkunsky, Kacoliris, & Bettina, 2014). These mountains belong to the Pampa eco-region, considered a maximum priority for conservation due to its great alteration, biological uniqueness, and the absence of protected areas (Bilenca & Miñarro, 2004). The Pampa eco-region has been predicted to have the highest suitability for *Bd* (Ghirardi et al., 2011). However, the presence of *Bd* has not yet been studied in these particular habitats of highland grassland.

The threatened *Melanophryniscus* populations are a high conservation priority (Vaira et al., 2012; Zank, Becker, Abadie, Maneyro, & Borges-Martins, 2014). In the next decades the populations of Tandilean Red-belly toad are predicted to lose climatic suitability in more than 60% of their present range (Zank et al., 2014). The fragmentation and modification of natural grasslands is causing mortality, affecting the dispersal of toads and the connection between reproductive ponds (Cairo & Zalba, 2007). Several studies demonstrated that abiotic factors and habitat loss can affect *Bd*-amphibian dynamics (Longo, Burrowes, & Joglar, 2010; Piotrowski, Annis, & Longcore, 2004; Rohr, Raffela, Romansic, McCallumb, & Hudson, 2008). Under scenarios of climate change and habitat fragmentation, *Bd* infections would represent an additional serious threat to the Tandilean Red-belly toad populations. Therefore, detection and quantification of *Bd* in remnants of highland grasslands will contribute to establish conservation strategies.

We surveyed temporary ponds in highland grasslands of the Tandilean Mountains (Fig. 1). We captured 32 adult toads in 2 different sites (Sierra del Tigre and Las Ánimas). Toads were captured by using visual-encounter surveys during 2 reproductive seasons (2012 and 2013). We identified each individual by photographs to prevent taking multiple samples from the same individual. We collected tissue from toads by swabbing (10 strokes) the ventral pelvic-patch region, thighs, and ventral surface (palmar) of hands and feet. DNA extraction from swab samples was done using 50 μl of PrepMan Ultra (Hyatt et al., 2007). Detection and quantification of *Bd* was done with the Taqman PCR method in an Applied Biosystems 7500 Real-Time PCR system according to Boyle, Boyle, Olsen, Morgan, and Hyatt (2004). Infection intensity was calculated as the number of *Bd* zoospore genomic equivalents in each swab sample.

Overall, 11 of 32 (35.5%) toads were *Bd*-positive. The infection was detected in both study sites. Infected individuals showed *Bd*-zoospore genomic equivalents ranging from 0.34 to 915 (mean = 103.2; SE = 253.5). All individuals examined were apparently healthy, and no sick, dying, or dead individuals were found.

We report the first record of chytridiomycosis on *M.* aff. *montevidensis* in the highland grasslands of the Tandilean Mountains, where at least 5 other species of amphibians occur. While this is the only threatened species, *Bd* could be affecting other species.

The decline of Red-belly toad is well documented. Populations from Ventania Mountains have been predicted to become extinct in 100 years (Cairo, 2010), while populations near Tandil City reported a decline and a complete extinction in some areas where this toad used to be abundant (Cortelezzi, com. pers.). Since these populations appear to be experiencing mostly sublethal levels of infection, we cannot assume a direct link between

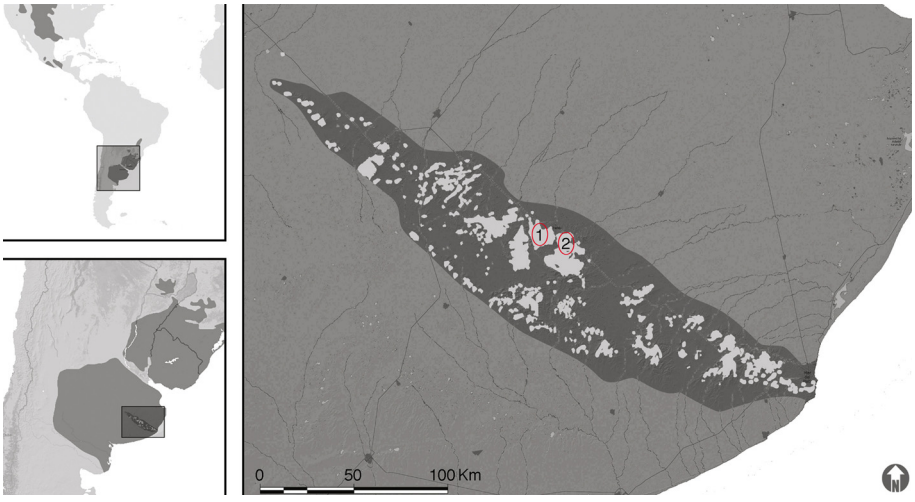

Figure 1. Study areas in the grassland relicts of Buenos Aires province (Argentina) where the amphibian surveys were performed. 1, Las Ánimas; 2, Sierra del Tigre Natural Reserve. Darker gray surface represents the Tandilean highland grasslands.

the population decline and *Bd* disease. But other studies have shown that amphibians persisting with *Bd* have significantly lower survival probabilities, and can die from chytridiomycosis (Longo & Burrowes, 2010; Longo, Ossiboff, Zamudio, & Burrowes, 2013).

Although this study presents *Bd* infection as another plausible cause for Red-belly toad population decline, the individual contribution of the disease in a multiple-causal scenario remains a challenge in need of investigation. The variation in distribution and prevalence of *Bd* across the fragmented landscape should be assessed taking into account an amphibian community perspective, in order to define the status and trend of chytridiomycosis in this relict of highland grasslands.

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